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Structure activity relationship of caffeic acid phenethyl ester analogs as new 5-lipoxygenase inhibitors.

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Abstract

Leukotrienes (LTs) are a class of lipid mediators implicated in numerous inflammatory disorders. Caffeic acid phenethyl ester (CAPE) possesses potent anti-LTs activity through the inhibition of 5-lipoxygenase (5-LO), the key enzyme in the biosynthesis of LTs. In this study, we describe the design and synthesis of CAPE analogs as radical scavengers and 5-LO inhibitors. Caffeic esters bearing propargyl and

allyl linkers between the caffeoyl and aryl moieties (**4a-i** and **5a-i**, respectively) were synthesized by Sonogashira and Heck cross coupling reactions to probe the effects of flexibility and aryl substitution on 5-LO inhibition. Caffeoyl alcohol and ethers (**6**, **7a-b**) as well as caffeoyl aldehyde and ketones (**8a-e**) were synthesized to elucidate the importance of the ester linkage for inhibitory activity. All tested compounds proved to be good radical scavengers (IC_{50} of 10 – 30 μM). After preliminary anti-LTs activity screening in HEK293 cell models, 5-LO inhibition potential of selected compounds was determined in human polymorphonuclear leukocytes (PMNL). Most screened compounds outperformed CAPE **3** in concentration-dependent assays on PMNL, with ester dimers **4i** and **5i** along with caffeoyl ethers **7a-b** being roughly 8-, 7- and 16-fold more potent than Zileuton, with IC_{50} values of 0.36, 0.43 and 0.18 μM, respectively.

Introduction

5-Lipoxygenase is the key enzyme in the transformation of arachidonic acid to leukotrienes (LTs), a class of eicosanoid inflammatory mediators which have been shown to play an important role in numerous inflammatory disorders such as asthma, atherosclerosis, and rheumatoid arthritis (1). Moreover, malignant cells of many cancers highly express 5-LO along with other related LTs biosynthesis enzymes, with anti-LTs therapy showing promising anticancer activity (1-5). In response to the need for drugs targeting the LTs biosynthesis pathway, numerous studies have been undertaken in order to develop efficient inhibitors of 5-LO (6,7). However, the only 5-LO inhibitor currently approved for human therapy, Zileuton (**1**) (fig.1), requires large and frequent dosing due to an unfavourable pharmacokinetic profile along with metabolites possessing hepatotoxic properties (1,8-10). Amongst potential alternative candidates for 5-LO inhibition are redox active compounds based on caffeic acid (**2**), notably caffeic acid phenethyl ester **3** (CAPE).

Figure 1. Structure of known 5-LO inhibitors Zileuton (**1**), caffeic acid (**2**), and CAPE (**3**).

CAPE (**3**) is a polyphenolic ester originally found in propolis, a resinous natural mixture of phytochemicals isolated from various plant sources by honeybees (11). Though propolis itself possesses a great deal of pharmacological activity, its variable and complex composition, which is largely dependent on the geographical location of collection, renders its standardisation and utilisation difficult (12,13). CAPE (**3**) has been found to possess differential cytotoxicity in assays of human normal and cancerous cell lines, preferentially targeting malignant cells. Additionally, it has shown anti-proliferative effects in a vast range of cancerous cell lines (14-17). Not limited to anti-cancerous properties, CAPE (**3**) also possesses anti-viral, immunomodulatory, anti-angiogenic, and antioxidant capacities, among others (13). Early work by Koshihara *et al.* showed that caffeic acid (**2**) and its methyl ester both possessed important anti-LTs activity (18,19). Based on these first studies, CAPE (**3**) was found to be an excellent inhibitor of plant lipoxygenase and has also been shown to reduce LTs formation in mouse peritoneal macrophages (20-22). Recent studies conducted in our laboratory have shown CAPE (**3**) to be significantly more potent than the clinical 5-LO inhibitor Zileuton **1** for the inhibition of LTs biosynthesis

in human polymorphonuclear leukocytes (PMNL, IC_{50} = 0.52 µM), which are important producers of the powerful chemoattractant leukotriene B_4 (23). CAPE (**3**) was also shown to be equipotent with Zileuton (**1**) in whole blood assays of 5-LO inhibition, an important consideration for the implementation of the inhibitor in *in vivo* systems.

Though it has now been established that CAPE (**3**) and other caffeic acid-based compounds have the potential to be potent inhibitors of 5-LO activity, little is known of the molecular basis for inhibition with these molecules. 5-LO derives its catalytic activity from a ferric iron atom present in its substrate-binding pocket (1). It is therefore assumed that caffeic acid-based compounds, which generally possess radical scavenging and antioxidant properties, derive their actions from redox interactions with this ferric iron, uncoupling the redox cycle necessary for LTs biosynthesis (6,7,24). Supporting this hypothesis is the finding that cinnamic acid analogs of CAPE (**3**) or of other caffeoyl-based 5-LO inhibitors, lacking the redox active phenolic hydroxyl groups, lose all 5-LO inhibitory activity (23,25,26). However, antioxidant activity is not sufficient to assure efficient 5-LO inhibition in complex systems: caffeic acid (**2**) and the amide analog of CAPE (**3**), which are roughly equipotent with CAPE (**3**) in radical scavenging assays, possess far lower inhibitory activity in PMNL and whole blood assays than CAPE (**3**) (23). Furthermore, assays of multivalent compounds showed that inhibitory activity of caffeates are not proportional to the number of caffeoyl groups present in a compound (25,26). Though most of the caffeoyl esters synthesized in these studies had excellent and similar 5-LO inhibition potential in HEK293 cell lysates, large variability in inhibition was found when performing assays on whole cells (25). Such differences in activity amongst structurally similar compounds possessing identical pharmacophores may indicate the important influence of structure and steric bulk on cell permeability and hydrophobic interactions.

In order to better comprehend the specific characteristics of CAPE (**3**) that confer efficient 5-LO inhibition, we hereby report the synthesis, radical scavenging capacity and 5-LO inhibition analysis of four novel series of CAPE analogs. In this study, focus is placed on the non-redox active terminus of the molecule, leaving the phenolic catechol group untouched (fig. 2).

Figure 2. Structures of proposed novel CAPE based 5-LO inhibitors and radical scavengers.

As can be seen in figure 2, modifications focus on the rigidification of the alkyl bridge of CAPE (**3**) through the synthesis of propargyl (**4a-i**) and allyl (**5a-i**) esters of caffeic acid by using Sonogashira or Heck cross coupling reactions with commercially available aryl iodides and the appropriate alkyne or alkene precursors. Terminal alkene and acetylene substituent groups were chosen for their electronic and steric properties. Additionally, as there has been very little investigation of CAPE ketone and ether analogs as 5-LO inhibitors, molecules where the ester group of CAPE (**3**) was replaced with an allyl ether/alcohol (**6**, **7a-b**) or a conjugated aldehyde/ketone group (**8a-e**) were also synthesized.

Methods and Materials

Experimental

General

All chemicals used were purchased from Aldrich (CA). Purification of compounds was carried out by silica gel circular chromatography (Chromatotron®, model 7924, Harrison Research) or by flash chromatography. TLC was run on silica gel coated aluminium sheets (SiliaPlate TLC, Silicycle®) with detection by UV light (254 nm, UVS-11, Mineralight® shortwave UV lamp). Melting points were obtained using a MELTEMP® (model 1001D) melting point apparatus. FTIR spectra were recorded on a Nicolet® Impact 400 spectrometer. NMR spectra were recorded on a Bruker® Avance III 400 MHz spectrometer. High-resolution mass measurements were performed on a Bruker® Doltonics' micrOTOF instrument in positive or negative electrospray.

General procedure I - Sonogashira coupling

Aryl iodide (2 mmol), $PdCl_2(PPh_3)_2$ (0.05 mmol, 35 mg) and CuI (0.1 mmol, 9.5 mg) are dissolved/suspended in 7 mL 1:1 DMF/NEt_3 and degassed by ultrasound for 5 minutes under a flow of Argon. **10** (1 mmol, 302 mg) is dissolved in 1 mL DMF and added dropwise over 15 minutes to the degassed solution with stirring at room temperature and under argon atmosphere. Progress is monitored by TLC (AcOEt/Hex). After complete consumption of starting material (about 4 h), the reaction mixture is diluted with 100 mL H_2O and extracted with three portions of 30 mL AcOEt. The organic fractions are washed with 2 x $NH_4Cl_{(sat)}$, 2 x H_2O, 2 x $NaCl_{(sat)}$, dried over $MgSO_4$, treated with activated charcoal, filtered and concentrated. The resulting oil is purified by flash chromatography or silica gel circular chromatography (Chromatotron® model 7924, Harrison Research). Eluent: AcOEt/Hex.

General procedure II - Heck coupling

To a stirred solution of $Pd(OAc)_2$ (0.05 mmol, 11.2 mg) and Ag_2CO_3 (0.6 mmol, 165 mg) in 7 mL benzene is added **12** (1 mmol, 304 mg) and aryl iodide (2 mmol). The resulting mixture is brought to reflux and left to react overnight. The benzene solution is then diluted with 60 mL AcOEt, washed with 2x $NH_4Cl_{(sat)}$, 2 x H_2O, 2 x $NaCl_{(sat)}$, dried over $MgSO_4$, treated with activated charcoal, filtered and concentrated. The resulting oil is purified by flash chromatography or silica gel circular chromatography (Chromatotron® model 7924, Harrison Research). Eluent: AcOEt/Hex).

General procedure III - Deacetylation of caffeoyl acetates

To a solution of the appropriate diacetylcaffeoyl derivative (0.25 mmol) in 2.5 mL anhydrous CH_2Cl_2 under N_2 is added 2.5 mL MeOH. To the resulting stirred solution is added guanidinium hydrochloride (1 mmol, 4 equivalents) followed by triethylamine (3 mmol, 12 eq.). After consumption of the diacetylated precursor as indicated by TLC (about 1.5 h), the reaction mixture is concentrated to remove excess NEt_3 and then diluted in 45 mL AcOEt. The organic phase is then washed with 2 x H_2O, 2 x $NH_4Cl_{(sat.)}$, 2 x $NaCl_{(sat)}$, treated with activated charcoal, dried over $MgSO_4$ and concentrated to give the expected pure caffeoyl derivative.

General Procedure IV – deprotection of tetrahydropyranyl ethers

To a stirred solution of the appropriate tetrahydropyranyl ether derivative (0.5 mmol) in 4 mL CH_2Cl_2 under argon is added 6 mL MeOH followed by pyridinium *p*-toluenesulfonate (25 mg, 0.1 mmol, 0.2 eq.). After complete consumption of starting material as indicated by TLC (about 4 h), the reaction mixture is concentrated and purified by flash chromatography ($MeOH/CH_2Cl_2$) to yield the appropriate catechol derivatives as crystalline solids or powder.

Diacetylcaffeic acid (9)

Compound **9** was prepared as previously described (26). Mp 190-192 ^{o}C. ^{1}H NMR (400 MHz, DMSO-*d*6, 25 °C), δ (ppm) = 7.70 (br s, 2H, H_{ar}), 7.55 (d, 1H, J = 18.6 Hz, CHC_{ar}), 7.20 (d, 1H, J = 8.6 Hz, H_{ar}), 6.5 (d, 1H, J = 18.6 Hz, CHCO), 2.3 (s, 6H, 2 x OAc). ^{13}C NMR (101 MHz, DMSO-*d*6, 25 °C), δ (ppm) = 168.2, 168.1, 167.4, 143.3, 142.3, 142.2, 133.1, 126.7, 124.1, 123.0, 120.3, 20.4, 20.3. HRMS m/z calc. for $C_{13}H_{12}O_6$ + (Na^+): 287.0526; found: 287.0522.

Prop-2-yn-1-yl (2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoate (10)

A mixture of **9** (1000 mg, 3.78 mmol), 3-4 drops of anhydrous DMF and 20 mL of thionyl chloride was heated at reflux for 3 h, under Ar. The excess thionyl chloride was then evaporated at reduced pressure, and the residue was dissolved in 5 mL of CH_2Cl_2. The resulting solution was added dropwise to a mixture of propargyl alcohol (848 mg, 15.1 mmol, 4 eq.) and pyridine (1255 mg, 15.87 mmol, 4.2 eq.) in 15 mL of CH_2Cl_2 at 0 ^{o}C under argon, then left to return to ambient temperature overnight. After concentration, the oily residue was diluted with 90 mL AcOEt and washed with 2 x 30 mL H_2O, 2 x 30 mL NH_4Cl_{sat}, 2 x $NaCl_{sat}$, dried over $MgSO_4$, filtered and concentrated. Compound **10** was obtained as white crystals with 84% yield (960 mg, 3.17 mmol) after silica gel circular chromatography (10-15% AcOEt/Hex); mp = 95-96 ^{o}C, R_f = 0.54 (40 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.70 (d, 1H, J = 16.0 Hz, $=CHC_{ar}$), 7.41 (d, 1H, J = 8.4 Hz, H_{ar}), 7.39 (s, 1H, H_{ar}), 7.25 (d, 1H, J = 8.4 Hz, H_{ar}), 6.43 (d, 1H, J = 16.0 Hz, =CHCOO), 4.84 (d, 2H, J = 2.4 Hz, CH_2CC), 2.54 (t, 1H, J = 2.4 Hz, CCH), 2.34 (s, 3H, OAc), 2.33 (s, 3H, OAc).

^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 168.04, 167.95, 165.65, 143.97, 143.72, 142.48, 133.01, 126.50, 124.00, 122.88, 118.19, 77.64, 75.04, 52.17, 20.65, 20.61. HRMS m/z calc. for $C_{16}H_{14}O_6$ + (Na^+): 325.0683; found: 325.0676.

3-phenylprop-2-yn-1-yl (2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoate (11b)

Following general procedure I with iodobenzene, compound **11b** was obtained with 57% yield as a white solid after silica gel circular chromatography (5-15% AcOEt/Hex); mp = 87-88 °C, R_f = 0.38 (30% AcOEt/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.72 (d, 1H, J = 16.0 Hz, =CHC_{ar}), 7.51-7.24 (m, 8H, H_{ar}), 6.46 (d, 1H, J = 16.0 Hz, =CHCOO), 5.07 (s, 2H, CH_2CC), 2.34 (s, 3H, OAc), 2.33 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 168.04, 167.95, 165.82, 143.80, 143.67, 142.48, 133.11, 131.93, 128.79, 128.31, 126.48, 123.99, 122.87, 122.14, 118.45, 86.65, 82.90, 53.08, 20.66, 20.61. HRMS m/z calc. for $C_{22}H_{18}O_6$ + (Na^+): 401.0996; found: 401.0984

3-[4-(3-{[(2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoyl]oxy}prop-1-yn-1-yl)phenyl]prop-2-yn-1-yl (2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoate (11i)

1,4-diiodobenzene (0.379 mmol, 125 mg), $PdCl_2(PPh_3)_2$ (0.019 mmol, 13.3 mg, 0.05 eq) and CuI (0.019 mmol, 3.6 mg, 0.05 eq) are dissolved/suspended in 7 mL 1:1 DMF/NEt_3 and degassed by ultrasound for 5 minutes under a flow of Argon. **10** (0.95 mmol, 286 mg, 2.5 eq) is dissolved in 1 mL DMF and added dropwise over 15 minutes to the degassed solution with stirring, at room temperature and under argon atmosphere. After 2h, TLC indicates complete consumption of starting material. The reaction mixture is diluted with 100 mL H_2O and extracted with three portions of 30 mL AcOEt. The organic fractions are washed with 2 x $NH_4Cl_{(sat)}$, 2 x H_2O, 2 x $NaCl_{(sat)}$, dried over $MgSO_4$, treated with activated charcoal, filtered and concentrated. The resulting oil is purified by silica gel circular chromatography (0.1 – 0.35 % MeOH/CH_2Cl_2). **11i** is obtained in 66 % yield as a white powder. mp = 192-194 (dec.), R_f = 0.20 (40 % AcOEt/Hex). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C), δ (ppm) = 7.75 - 7.71 (m, 6H, H_{ar} + =CHC_{ar}), 7.50 (s, 4H, H_{ar}), 7.34 (d, 2H, J = 8.5 Hz, H_{ar}), 6.74 (d, 2H, J = 16.0 Hz, =CHCOO), 5.05 (s, 4H, CH_2CC), 2.31 (s, 3H, OAc), 2.30 (s, 3H, OAc). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C), δ (ppm) = 168.65, 168.55, 165.81, 144.30, 144.15, 142.81, 133.22, 132.33, 127.65, 124.65, 123.83, 122.45, 118.72, 86.82, 85.60, 52.97, 20.85, 20.76. HRMS m/z calc. for $C_{38}H_{30}O_{12}$ + (Na^+): 701.1629; found: 701.1648.

prop-2-yn-1-yl (2E)-3-[3,4-dihydroxyphenyl]prop-2-enoate (4a)

Following general procedure III with **10**, compound **4a** was obtained with 73% yield as a pale brown powder, mp = 99 – 100 °C, R_f = 0.46 (5% MeOH/CH_2Cl). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C), δ (ppm) = 9.40 (br s, 2H, OH), 7.52 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 7.08 (s, 1H, H_{ar}), 7.04 (d, 1H, J = 8.2 Hz, H_{ar}), 6.77 (d, 1H, J = 8.2 Hz, H_{ar}), 6.31 (d, 1H, J = 15.9 Hz, =CHCOO), 4.79 (s, 2H, CH_2CC), 3.46 (s, 1H, CCH). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C), δ (ppm) = 166.24, 149.14, 146.62, 146.04, 125.80, 122.15, 116.18, 115.40, 113.37, 79.21, 78.01, 51.93. HRMS m/z calc. for $C_{12}H_{10}O_4$ + (Na^+): 241.0471; found: 241.0469.

prop-2-en-1-yl (2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoate (12)

A mixture of **9** (1036.0 mg, 3.92 mmol), 3-4 drops of anhydrous DMF and 20 mL of thionyl chloride was heated at reflux for 3 h, under Ar. The excess thionyl chloride was then evaporated, and the residue was dissolved in 5-10 mL of CH_2Cl_2. The solution was added dropwise to a mixture of allyl alcohol (905.2 mg, 15.59 mmol, 4 eq.), pyridine (1302.3 mg, 16.46 mmol, 4.2 eq.) and 5 mL of CH_2Cl_2 stirred at 0 ^oC under Ar, then left to return to ambient temperature overnight. After concentration, the oily residue was diluted with 90 mL AcOEt and washed with 2 x 30 mL H_2O, 2 x 30 mL NH_4Cl_{sat}, 2 x $NaCl_{sat}$, dried over $MgSO_4$, filtered and concentrated. Compound **12** obtained with 74 % yield (880.8 mg, 2.89 mmol) after silica gel circular chromatography (5-15% AcOEt in hexanes); white solid, mp = 78-80 ^oC, R_f = 0.29 (30% AcOEt/Hex). ^1H NMR (400 MHz, $CDCl_3$, 25 ^oC) δ (ppm): 7.67 (d, J = 16.0 Hz, 1H, =CHC_{ar}), 7.43 (d, J = 8.40 Hz, 1H, H_{ar}), 7.38 (s, 1H, H_{ar}), 7.24 (d, J = 8.3 Hz, 1H, H_{ar}), 6.43 (d, J = 16.0 Hz, 1H, =CHCO), 6.01 (m, 1H, $CH_2C\mathbf{H}=CH_2$), 5.39 (d, J = 17.25 Hz, 1H, $CH_2CH=C\mathbf{H}H$), 5.30 (d, J = 10.44 Hz, 1H, $CH_2CH=CH\mathbf{H}$), 4.73 (d, J = 5.40 Hz, 2H, $C\mathbf{H}_2CH=CH_2$), 2.34 (s, 3H, OAc), 2.33 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$, 25 ^oC) δ (ppm): 168.09, 168.01, 166.21, 143.52, 143.10, 142.44, 133.25, 132.14, 126.43, 123.96, 122.76, 119.06, 118.39, 65.35, 20.68,20.63. HRMS m/z calc. for $C_{16}H_{16}O_6$ + (Na^+): 327.0839; found: 327.0824.

(2E)-3-phenylprop-2-en-1-yl (2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoate (13b)

Following general procedure II with iodobenzene, compound **13b** was obtained with 65% yield as a yellow solid after silica gel circular chromatography (5-10% AcOEt/Hex); mp 76-77 ^oC, R_f = 0.26 (30% AcOEt/hexanes). ^1H NMR (400 MHz, $CDCl_3$, 25 ^oC) δ (ppm): 7.69 (d, 1H, J = 16.0 Hz, C=CHC_{ar}), 7.45–7.24 (m, 8H, H_{ar}), 6.73 (d, 1H, J = 15.9 Hz, $CH_2CH=C\mathbf{H}$), 6.45 (d, 1H, J = 16.0 Hz, =CHCO), 6.38 (dt, 1H, J = 15.9 Hz, 6.3 Hz, $CH_2C\mathbf{H}$=CH), 4.89 (d, 2H, J = 6.3 Hz, $C\mathbf{H_2}$CH=CH), 2.34 (s, 3H, OAc), 2.33 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$, 25 ^oC) δ (ppm): 168.10, 168.02, 166.35, 143.54, 143.16,142.45, 136.20, 134.35, 133.27, 128.63, 128.12, 126.66, 126.45, 123.97, 123.12, 122.78, 119.10, 65.30, 20.68, 20.64. HRMS m/z calc. for $C_{22}H_{20}O_6$ + (Na^+): 403.1152; found: 403.1144.

(2E)-4-{4-[(1E)-3-{[(2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoyl]oxy}prop-1-en-1-yl]phenyl}but-2-en-1-yl (2E)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoate (13i)

To a stirred solution of $Pd(OAc)_2$ (0.075 mmol, 16.8 mg, 0.05 eq) and Ag_2CO_3 (0.9 mmol, 248 mg, 0.6 eq) in 7 mL benzene is added **12** (1.5 mmol, 456 mg, 1 eq) and 1,4-diiodobenzene (0.66 mmol, 217 mg, 0.44 eq). The resulting mixture is brought to reflux and left to react overnight. The benzene solution is then diluted with 60 mL AcOEt, washed with 2x $NH_4Cl_{(sat)}$, 2 x H_2O, 2 x $NaCl_{(sat)}$, dried over $MgSO_4$, treated with activated charcoal, filtered and concentrated. The resulting oil is purified by silica gel circular chromatography (0 – 0.5 % MeOH/CH_2Cl_2). **13i** was obtained with 57% yield as a yellow solid. mp = 142-144 ^oC, R_f = 0.59 (2% MeOH/CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$, 25 ^oC), δ (ppm) = 7.68 (d, 2H, J = 16.0 Hz, =CHC_{ar}), 7.43 (dd, 2H, J = 8.4 Hz, 2.1 Hz, H_{ar}), 7.40 (s, 4H, H_{ar}), 7.39 (d, 2H, J = 2.1 Hz, H_{ar}), 7.24 (d, 2H, J = 8.4 Hz, H_{ar}), 6.70 (d, 2H, J = 15.9 Hz, $CH_2CH=C\mathbf{H}$), 6.44 (d, 2H, J = 16.0 Hz, =CHCOO), 6.39 (dt, 2H, J = 15.9

Hz, 6.4 Hz, CH_2CH=CH), 4.89 (d, 4H, J = 6.4 Hz, CH_2CH=CH), 2.33 (s, 3H, OAc), 2.32 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$, 25 ^{o}C), δ (ppm) = 168.07, 167.99, 166.31, 143.54, 143.18, 142.45, 136.00, 133.80, 133.26, 126.93, 126.43, 123.96, 123.32, 122.78, 119.07, 65.25, 20.67, 20.63. HRMS m/z calc. for $C_{38}H_{34}O_{12}$ + (Na^+): 705.1942; found: 705.1946.

(*E*)-methyl 3-(3,4-dihydroxyphenyl)acrylate (14)

To a stirred solution of **2** (5400 mg, 30 mmol) dissolved in 100 mL MeOH is added 1 mL conc. H_2SO_4 and the mixture is heated to reflux for 4h. After complete consumption of starting material, the reaction mixture is concentrated to 30 mL and diluted with 350 mL AcOEt. The resulting solution is washed with 2 x $NaHCO_{3(sat.)}$, 1 x water, 1 x brine, dried over $MgSO_4$, filtered and concentrated. Compound **14** was obtained with 89 % yield (5543 mg, 26.6 mmol) as a light yellow crystals; mp = 153 – 154 ^{o}C, Rf = 0.60 (7 % MeOH/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 9.37 (br s, 2H, OH), 7.49 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 7.06 (d, 1H, J = 1.8 Hz, H_{ar}), 7.00 (dd, 1H, J = 8.2 Hz, 1.8 Hz, H_{ar}), 6.77 (d, 1H, J = 8.2 Hz, H_{ar}), 6.28 (d, 1H, J = 15.9 Hz, =CHCO), 3.69 (s, 1H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 167.48, 148.89, 146.03, 145.63, 125.94, 121.89, 116.18, 115.22, 114.14, 51.68. HRMS m/z calc. for $C_{10}H_{10}O_4$ + (H^+): 195.0652; found: 195.0643.

(*E*)-methyl 3-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)acrylate (15)

To a stirred solution of **14** (4164 mg, 20 mmol) dissolved in 50 mL dihydropyran is added pyridinium p-toluenesulfonate (251 mg, 1mmol, 0.05 eq.) and the mixture is heated to 60 ^{o}C on a water bath for 90 minutes. After complete consumption of starting material, excess dihydropyran is removed under reduced pressure and the resulting oil is dissolved in 200 mL CH_2Cl_2. The organic solution is washed with 2 x $NaHCO_{3(sat.)}$, 1 x water, 1 x brine, treated with activated charcoal, filtered and concentrated. Compound **15**, a mixture of diastereoisomers, is obtained as a translucent oil with 88 % yield (6624 mg, 17.6 mmol) after flash chromatography (6 % AcOEt/Hex); Rf = 0.52 (30 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.63 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 7.35 (br s, 1H, H_{ar}), 7.16 (br s, 2H, H_{ar}), 6.32 (d, 1H, J = 15.9 Hz, =CHCO), 5.51 (m, 1H, OCHO), 5.46 (m, 1H, OCHO), 4.05 – 3.92 (m, 2H, CH_2O), 3.81 (s, 1H, CH_3), 3.68 – 3.62 (m, 2H, CH_2O), 2.07 – 1.86 (m, 6H, THP), 1.78 – 1.56 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 167.68, 149.70, 149.46, 147.52, 147.30, 144.74, 144.71, 128.82, 128.69, 123.62, 123.47, 117.98, 117.78, 117.60, 117.50, 116.04, 115.99, 97.91, 97.43, 97.22, 96.82, 62.01, 61.93, 61.86, 61.75, 51.59, 30.36, 30.32, 30.23, 30.20, 25.27, 25.20, 18.62, 18.52, 18.37. HRMS m/z calc. for $C_{20}H_{26}O_6$ + (Na^+): 385.1622; found: 385.1604.

(*E*)-3-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)prop-2-en-1-ol (16)

To a stirred solution of **15** (2636 mg, 7 mmol) in 40 mL anhydrous toluene under argon at -78 °C is added dropwise diisobutylaluminium hydride (15.3 ml as a 1M solution in toluene, 15.3 mmol, 2.2 eq.) over the course of 30 minutes. The reaction is stirred at -78 °C for 90 minutes after which 10 mL of MeOH is slowly added to destroy excess DIBAL-H. After having returned to ambient temperature, 50 mL of saturated potassium sodium tartrate solution is added to the organic solution and vigorously stirred for 4h. The organic and aqueous phases are then separated and the aqueous phase is extracted with 3 x 30 mL AcOEt. The pooled organic fractions are washed with 2 x $NH_4Cl_{(sat.)}$), dried over $MgSO_4$, filtered and concentrated to yield compound **16**, a mixture of diastereoisomers, in quantitative yields as a translucent oil; R_f = 0.21 (30 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.21 (m, 1H, H_{ar}), 7.09 (d, 1H, J = 8.34, H_{ar}), 6.99 (d, 1H, J = 8.34, H_{ar}), 6.53 (d, 1H, J = 15.8 Hz, =CHC_{ar}), 6.25 (dt, 1H, J = 15.8 Hz, 5.9 Hz, =C**H**CH_2O), 5.47 – 5.44 (m, 2H, OCHO), 4.29 (d, 2H, CH_2O), 4.06 – 3.97 (m, 2H, CH_2O), 3.67 – 3.61 (m, 2H, CH_2O), 2.07 – 1.84 (m, 6H, THP), 1.78 – 1.53 (m, 7H, THP + OH). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 147.59, 147.36, 147.14, 131.54, 131.38, 130.99, 130.96, 127.18, 121.23, 121.09, 118.13, 116.64, 116.31, 97.81, 97.64, 97.37, 97.20, 63.82, 61.97, 61.93, 61.80, 61.75, 30.42, 30.37, 30.34, 30.30, 25.32, 25.29, 18.68, 18.64, 18.55, 18.50. HRMS m/z calc. for $C_{19}H_{26}O_5$ + (Na^+): 357.1672; found: 157.1650.

(*E*)-2,2'-((4-(3-(benzyloxy)prop-1-en-1-yl)-1,2-phenylene)bis(oxy))bis(tetrahydro-2*H*-pyran) (17a)

To a suspension of NaH (46 mg of a 60 % NaH dispersion in mineral oil, 1.15 mmol, 2.1 eq.) in anhydrous DMF at 0 °C is added dropwise **16** (191 mg, 0.55 mmol) dissolved in 1 mL DMF and the mixture is left to react for 30 minutes. Benzyl bromide (196 mg, 1.15 mmol, 2.1 eq) dissolved in 1 mL DMF is then added dropwise to the alcoxide solution while maintaining the temperature of the mixture below 5 °C, and the solution is stirred for an additional 2h and then quenched by slow addition of 0.1 mL MeOH. The resulting solution is diluted to 75 mL with H_2O and then extracted 5 x 15 mL AcOEt. The organic phase is washed with 2 x H_2O, 1 x brine, dried over $MgSO_4$ and concentrated. Compound **17a**, a mixture of diastereoisomers, is obtained as a thick translucent oil with 70 % yield (167 mg, 0.38 mmol) after silica gel circular chromatography (6 % AcOEt/Hex); Rf = 0.63 (20 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.40 – 7.36 (m, 3H, H_{ar}), 7.32 – 7.28 (m, 2H, H_{ar}), 7.23 (t, 1H, J = 1.9 Hz, H_{ar}), 7.10 (dd, 1H, J = 8.3 Hz, 2.2 Hz), 7.00 (dt, 1H, J = 8.3 Hz, 1.9 Hz, H_{ar}), 6.56 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 6.22 (dt, 1H, J = 15.9 Hz, 5.9 Hz, =C**H**CH_2O), 5.47 – 5.45 (m, 2H, OCHO), 4.58 (s, 2H, OCH_2Ph), 4.19 (d, 2H, J = 5.9 Hz, =CHC**H$_2$**O), 4.07 – 3.97 (m, 2H, CH_2O), 3.68 – 3.45 (m, 2H, CH_2O), 2.13 – 1.83 (m, 6H, THP), 1.79 – 1.58 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 147.57, 147.36, 147.15, 138.35, 132.39, 132.36, 131.59, 128.41, 128.36, 127.82, 124.66, 124.60, 121.27, 121.13, 118.42, 118.10, 116.68, 116.35, 97.80, 97.63, 97.36, 97.21, 72.03, 70.87, 61.96, 61.93, 61.80, 61.76, 30.43, 30.38, 30.35, 30.31, 25.33, 25.30, 18.69, 18.65, 18.56, 18.51. HRMS m/z calc. for $C_{26}H_{32}O_5$ + (Na^+): 447.2142; found: 447.2114.

(*E*)-2,2'-((4-(3-(3-phenylpropoxy)prop-1-en-1-yl)-1,2-phenylene)bis(oxy))bis(tetrahydro-2*H*-pyran) (17b)

To a suspension of NaH (63 mg of a 60 % NaH dispersion in mineral oil, 1.58 mmol, 2.1 eq.) in anhydrous DMF at 0 °C is added dropwise **16** dissolved in 1 mL DMF and the mixture is left to react for 30 minutes. (3-bromopropyl)benzene (314 mg, 1.58 mmol, 2.1 eq) dissolved in 1 mL DMF is then added dropwise to the alcoxide solution while maintaining the temperature of the mixture below 5 °C, and the solution is stirred for an additional 2h and then quenched by slow addition of 0.1 mL MeOH. The resulting solution is diluted to 75 mL with H_2O and then extracted 5 x 15 mL AcOEt. The organic phase is washed with 2 x H_2O, 1 x brine, dried over $MgSO_4$ and concentrated. Compound **17b**, a mixture of diastereoisomers, is obtained as a thick translucent oil with 70 % yield (167 mg, 0.38 mmol) after silica gel circular chromatography (5 % AcOEt/Hex); Rf = 0.65 (20 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.32 – 7. 30 (m, 2H, H_{ar}), 7.23 – 7.18 (m, 4H, H_{ar}), 7.10 (dd, 1H, J = 8.4 Hz, 2.3 Hz), 7.00 (dt, 1H, J = 8.4 Hz, 2.0 Hz, H_{ar}), 6.53 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 6.19 (dt, 1H, J = 15.9 Hz, 6.1 Hz, =C**H**CH_2O), 5.48 – 5.44 (m, 2H, OCHO), 4.12 (d, 2H, J = 6.1 Hz, =CHC**H$_2$**O), 4.07 – 3.98 (m, 2H, CH_2O), 3.67 – 3.60 (m, 2H, CH_2O), 3.50 (t, 2H, J = 6.4 Hz, OC**H$_2$**CH_2CH_2Ph), 2.74 (t, 2H, J = 6.4 Hz, OCH_2CH_2C**H$_2$**Ph), 2.13 – 1.84 (m, 8H, THP + OCH_2C**H$_2$**CH_2Ph), 1.80 – 1.68 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 147.57, 147.35, 147.32, 147.10, 142.02, 132.05, 132.02, 131.65, 131.49, 128.50, 125.75, 124.92, 124.86, 121.25, 121.10, 118.43, 118.10, 116.67, 116.33, 97.79, 97.64, 97.35, 97.21, 71.55, 69.40, 61.95, 61.93, 61.79, 61.76, 32.40, 31.38, 30.43, 30.39, 30.35, 30.31, 25.33, 25.30, 18.68, 18.66, 18.56, 18.52. HRMS m/z calc. for $C_{28}H_{36}O_5$ + (Na^+): 475.2455; found: 475.2433.

(*E*)-4-(3-hydroxyprop-1-en-1-yl)benzene-1,2-diol, caffeoyl alcohol (6)

Following general procedure IV with **16**, compound **6** was obtained with 70 % yield as white crystals after silica gel circular chromatography (3 - 4 % MeOH/CH_2Cl_2); mp = 0.45 (7.5 % MeOH/CH_2Cl_2), Rf = 0.45 (7.5 % MeOH/CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C), δ (ppm) = 8.90 (br s, 2H, OH), 6.81 (br s, 1H, H_{ar}), 6.67 (br s, 2H, H_{ar}), 6.34 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 6.04 (dt, 1H, J = 15.9 Hz, 5.4 Hz, =C**H**CH_2OH), 4.73 (br s, 1H, OH), 4.05 (d, 1H, J = 5.4 Hz, C**H$_2$**OH). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C), δ (ppm) = 145.74, 145.47, 129.52, 128.92, 127.46, 118.38, 116.06, 113.49, 62.18. HRMS m/z calc. for $C_9H_{10}O_3$ + (H^+): 149.0597; found: 149.0586.

(*E*)-3-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)-*N*-methoxy-*N*-methylacrylamide (18)

Compound **15** (1130 mg, 3 mmol) and N,O-dimethylhydroxylamine hydrochloride (454 mg, 4.65 mmol, 1.55 eq.) are dissolved/suspended in 15 mL anhydrous THF under argon and placed at -20 °C with agitation. Isopropyl magnesium chloride (4.5 mL as a 2M solution in THF, 9 mmol, 3 eq.) is then added dropwise over 10 minutes while keeping the reaction temperature below –10 °C. After 25 minutes, the reaction is quenched by slow addition of 5 mL of 5M NH_4Cl and agitation is continued for another 10 minutes. The resulting mixture is diluted with 50 mL 5M NH_4Cl and extracted with 3 x 30 mL AcOEt. The

organic phase is then dried over $MgSO_4$, filtered and concentrated. Compound **18**, a mixture of diastereoisomers, is obtained as a translucent oil with 87 % yield (1056 mg, 2.6 mmol) after silica gel circular chromatography (20 - 30 % AcOEt/Hex); Rf = 0.16 (30 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.67 (d, 1H, J = 15.9 Hz, =CHC_{ar}), 7.37 (br s, 1H, H_{ar}), 7.21 (m, 1H, H_{ar}), 7.15 (m, 1H, H_{ar}), 6.90 (d, 1H, J = 15.9 Hz, =CHCO), 5.52 – 5.45 (m, 2H, OCHO), 4.06 – 3.93 (m, 2H, CH_2O), 3.77 (s, 1H, OCH_3), 3.65 – 3.61 (m, 2H, CH_2O), 3.31 (s, 3H, NCH_3), 2.11 – 1.88 (m, 6H, THP), 1.77 – 1.63 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 167.24, 149.35, 149.12, 147.39, 147.18, 143.35, 143.31, 129.68, 129.55, 123.45, 123.29, 118.38, 117.99, 117.81, 117.57, 114.12, 114.06, 98.06, 97.57, 97.26, 96.87, 62.15, 61.98, 61.93, 61.84, 61.75, 32.54, 30.40, 30.35, 30.25, 30.22, 25.28, 25.22, 18.76, 18.64, 18.55, 18.40. HRMS m/z calc. for $C_{21}H_{29}NO_6$ + (H^+): 392.2068; found: 392.2039.

(*E*)-3-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)acrylaldehyde, diacetylcaffealdehyde (19a)

To a vigorously stirred solution of compound **16** (118 mg, 0.34 mmol) in 8 mL CH_2Cl_2 under argon is added MnO_2 (300 mg, 3.4 mmol, 10 eq.) and the mixture is left to react overnight. After 16h the mixture is filtered on a pad of celite and concentrated. Compound **19a**, a mixture of diastereoisomers, is obtained as a translucent oil with 53 % yield (62 mg, 0.18 mmol) after silica gel circular chromatography (7.5 % AcOEt/Hex); Rf = 0.24 (20 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 9.67 (d, 1H, J = 7.8 Hz, CHO), 7.41 (d, 1H, J = 15.8 Hz, =CHC_{ar}), 7.39 (s, 1H, H_{ar}), 7.23 - 7.18 (m, 2H, H_{ar}), 6.63 (dd, 1H, J = 15.8 Hz, 7.8 Hz, =CHCHO), 5.55 (m, 1H, OCHO), 5.47 (m, 1H, OCHO), 4.05 - 3.91 (m, 2H, CH_2O), 3.67 - 3.63 (m, 2H, CH_2O), 2.10 - 1.88 (m, 6H, THP), 1.79 - 1.62 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 193.68, 152.90, 152.85, 150.55, 150.30, 147.60, 147.38, 128.35, 128.22, 127.20, 127.16, 124.20, 124.03, 118.35, 117.96, 117.63, 117.37, 97.96, 97.47, 97.13, 96.74, 62.05, 61.96, 61.91, 61.78, 30.35, 30.30, 30.18, 30.16, 25.24, 25.16, 18.60, 18.50, 18.46, 18.32. HRMS m/z calc. for $C_{19}H_{24}O_5$ + (Na^+): 355.1516; found: 355.1498.

(*E*)-3-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)-1-phenylprop-2-en-1-one (19b)

To a stirred solution of **18** (285 mg, 0.70 mmol) dissolved in 7 mL anhydrous THF at 0 °C and under argon is slowly added phenyl magnesium bromide (1.76 mL as a 1M solution in THF, 1.76 mmol, 2.5 eq.) over 10 minutes while keeping the solution at 0 °C. After 45 minutes, the reaction is quenched by slow addition of 7 mL of 5M NH_4Cl and agitation is continued for another 10 minutes. The resulting mixture is extracted with 3 x 30 mL CH_2Cl_2. The organic phase is then dried over $MgSO_4$, filtered and concentrated. Compound **19b**, a mixture of diastereoisomers, is obtained as a thick yellow oil with 70 % yield (205 mg, 0.49 mmol) after silica gel circular chromatography (12 % AcOEt/Hex); Rf = 0.37 (20 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 8.02 (d, 2H, J = 7.3 Hz, H_{ar}), 7.45 (d, 1H, J = 15.7 Hz, =CHC_{ar}), 7.60 (t, 1H, J = 7.3 Hz, H_{ar}), 7.51 (t, 2H, J = 7.3 Hz, H_{ar}), 7.42 (br s, 1H, H_{ar}), 7.40 (d, 1H, J = 15.7 Hz, =CHCO), 7.30 (m, 1H, H_{ar}), 7.19 (m, 1H, H_{ar}), 5.56 (m, 2H, OCHO), 4.08 – 3.94 (m, 2H, CH_2O), 3.69 – 3.63 (m, 2H, CH_2O), 2.10 (m, 6H, THP), 1.80 – 1.59 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 190.73, 150.03, 149.79, 147.53, 147.31, 144.99, 144.94, 138.49, 132.55, 129.29, 129.15, 128.56, 128.47, 124.25,

124.11, 120.60, 120.55, 118.27, 117.85, 117.74, 117.47, 97.94, 97.43, 97.21, 96.81, 62.08, 61.96, 61.92, 61.78, 30.40, 30.35, 30.23, 30.21, 25.29, 25.21, 25.20, 18.65, 18.55, 18.52, 18.38. HRMS m/z calc. for $C_{25}H_{28}O_5$ + (Na^+): 431.1829; found: 431.1817.

(*E*)-4-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)-1-phenylbut-3-en-2-one (19c)

To a stirred solution of **18** (405 mg, 1 mmol) dissolved in 8 mL anhydrous THF at 0 °C and under argon is slowly added benzyl magnesium chloride (2.5 mL as a 1M solution in THF, 2.5 mmol, 2.5 eq.) over 10 minutes while keeping the solution at 0 °C. After 45 minutes, the reaction is quenched by slow addition of 7 mL of 5M NH_4Cl and agitation is continued for another 10 minutes. The resulting mixture is extracted with 3 x 30 mL CH_2Cl_2. The organic phase is then dried over $MgSO_4$, filtered and concentrated. Compound **19c**, a mixture of diastereoisomers, is obtained as a thick translucent oil with 41 % yield (174 mg, 0.41 mmol) after silica gel circular chromatography (12 % AcOEt/Hex); Rf = 0.42 (25 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.57 (d, 1H, J = 16.0 Hz, =CHC_{ar}), 7.40 – 7.31 (m, 5H, H_{ar}), 7.29 – 7.26 (m, 3H, H_{ar}), 7.18 – 7.13 (m, 2H, H_{ar}), 6.67 (d, 1H, J = 16.0 Hz, =CHCO), 5.51 (m, 1H, OCHO), 5.46 (m, 1H, OCHO), 4.52 – 3.91 (m, 4H, CH_2O + $PhCH_2$), 3.70 – 3.61 (m, 2H, CH_2O), 2.07 – 1.89 (m, 6H, THP), 1.75 – 1.61 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 197.37, 149.99, 149.75, 147.49, 147.27, 143.48, 143.43, 134.68, 129.48, 128.77, 128.72, 128.63, 128.58, 127.68, 126.99, 126.90, 124.04, 123.92, 123.88, 118.26, 117.85, 117.70, 117.43, 97.92, 97.42, 97.18, 96.77, 62.06, 61.94, 61.91, 61.76, 48.06, 30.36, 30.31, 30.21, 30.18, 25.27, 25.19, 18.64, 18.53, 18.49, 18.35. HRMS m/z calc. for $C_{26}H_{30}O_5$ + (Na^+): 445.1985; found: 445.1963.

(*E*)-1-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)-5-phenylpent-1-en-3-one (19d)

To a stirred solution of **18** (285 mg, 0.70 mmol) dissolved in 7 mL anhydrous THF at 0 °C and under argon is slowly added phenylethyl magnesium chloride (2.5 mL as a 1M solution in THF, 2.5 mmol, 2.5 eq.) over 10 minutes while keeping the solution at 0 °C. After 45 minutes, the reaction is quenched by slow addition of 7 mL of 5M NH_4Cl and agitation is continued for another 10 minutes. The resulting mixture is extracted with 3 x 30 mL CH_2Cl_2. The organic phase is then dried over $MgSO_4$, filtered and concentrated. Compound **19d**, a mixture of diastereoisomers, is obtained as a thick translucent oil with 51 % yield (212 mg, 0.51 mmol) after silica gel circular chromatography (12 % AcOEt/Hex); Rf = 0.68 (30 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.49 (d, 1H, J = 16.1 Hz, =CHC_{ar}), 7.37 – 7.20 (m, 6H, H_{ar}), 7.16 (m, 2H, H_{ar}), 6.63 (d, 16.1 Hz, =CHCO), 5.52 (m, 1H, OCHO), 5.47 (m, 1H, OCHO), 4.06 – 3.92 (m, 2H, CH_2O), 3.68 – 3.63 (m, 2H, CH_2O), 3.04 – 3.00 (m, 4H, CH_2CH_2), 2.07 – 1.85 (m, 6H, THP), 1.78 – 1.59 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 199.37, 149.90, 149.66, 147.55, 147.33, 142.77, 142.73, 141.33, 128.82, 128.68, 128.50, 128.47, 128.40, 128.34, 126.08, 125.84, 124.74, 124.69, 123.95, 123.80, 118.04, 117.76, 117.64, 117.48, 97.88, 97.40, 97.21, 96.80, 62.02, 61.95, 61.87, 61.77, 42.24, 30.42, 30.37, 30.32, 30.26, 30.22, 30.19, 25.27, 25.20, 25.19, 18.60, 18.50, 18.37. HRMS m/z calc. for $C_{27}H_{32}O_5$ + (K^+): 475.1881; found: 475.1874.

(*E*)-1-(3,4-bis((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)-6-phenylhex-1-en-3-one (19e)

To 3 mL anhydrous THF is added acid washed magnesium shavings (80 mg, 3.3 mmol, 1.1 eq./bromide) followed by a small crystal of iodine. A small portion (about 20 % of total mass) of (3-bromopropyl)benzene (597 mg, 3 mmol, 3 eq.) is added dropwise to the magnesium suspension in order to initiate formation of the organomagnesium reagent. Once Grignard reagent formation begins (as indicated by light boiling) the remaining (3-bromopropyl)benzene is added slowly in order to maintain a gentle reflux. Once the Grignard reagent formation is complete, the resulting solution of (3-bromomagnesiumpropyl)benzene in THF is added dropwise to a stirred solution of **18** (405 mg, 1 mmol) dissolved in 7 mL anhydrous THF at 0 ^{o}C and under argon over 10 minutes while keeping the solution at 0 ^{o}C. After 45 minutes, the reaction is quenched by slow addition of 7 mL of 5M NH_4Cl and agitation is continued for another 10 minutes. The resulting mixture is extracted with 3 x 30 mL CH_2Cl_2. The organic phase is then dried over $MgSO_4$, filtered and concentrated. Compound **19e**, a mixture of diastereoisomers, is obtained as a thick yellow oil with 22 % yield (104 mg, 0.22 mmol) after silica gel circular chromatography (12 % AcOEt/Hex); Rf = 0.71 (30 % AcOEt/Hex). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C), δ (ppm) = 7.45 (d, 1H, J = 16.1 Hz, =CHC_{ar}), 7.36 – 7.29 (m, 3H, H_{ar}), 7.24 – 7.20 (m, 3H, H_{ar}), 7.17 (m, 2H, H_{ar}), 6.61 (d, 1H, J = 16.1 Hz, =CHCO), 5.52 (m, 1H, OCHO), 5.48 (m, 1H, OCHO), 4.03 – 3.96 (m, 2H, CH_2O), 3.69 – 3.61 (m, 2H, CH_2O), 2.73 – 2.66 (m, 4H, COC**H$_2$**CH_2C**H$_2$**Ph), 2.11 – 1.85 (m, 8H, $COCH_2$C**H$_2$**CH_2Ph + THP), 1.81 – 1.60 (m, 6H, THP). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C), δ (ppm) = 200.25, 149.84, 149.61, 147.56, 147.33, 142.50. 142.46, 141.75, 128.89, 128.75, 128.55, 128.39, 125.93, 124.81, 124.76, 123.92, 123.76, 118.00, 117.79, 117.59, 117.50, 97.85, 97.37, 97.22, 96.81, 62.01, 61.95, 61.86, 61.77, 39.76, 35.20, 30.38, 30.33, 30.23, 30.20, 25.87, 25.29, 25.21, 18.60, 18.51, 18.38. HRMS m/z calc. for $C_{28}H_{34}O_5$ + (Na^+): 473.2298; found: 473.2284.

Antiradical activity assay

The radical scavenging activity of test compounds was measured as previously described using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a stable radical (20) with slight modifications. Particular care was taken in the preparation of the control (DPPH reagent + ethanol as a diluent without test compounds). Controls with O.D. of 0.350-0.360 at 520nm were deemed acceptable to avoid variations in IC_{50} calculations. 1 ml of DPPH in ethanol (60 mM) was mixed with 1 mL of the test compounds at the indicated concentrations or their diluent (ethanol). Each mixture was then shaken vigorously and kept in the dark for 30 min at room temperature. The absorbance of DPPH at 520 nm was then measured. The radical scavenging activity was expressed in terms of % inhibition of DPPH absorbance:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ test})/A \text{ control})] \times 100$$

where "A control" is the absorbance of the control (DPPH solution without test compound) and "A test" is the absorbance of the test sample (DPPH solution plus compound). Data are expressed as means ± SD of 3 independent experiments, each performed in triplicate (table 1). IC_{50} values were calculated from a

sigmoidal concentration-response curve-fitting model with a variable slope on GraphPad Prism 5 software (GraphPad Software, San Diego, California).

5-LO product biosynthesis assays in HEK293 cells

HEK293 cells stably co-transfected with a pcDNA3.1 vector expressing 5-LO and a pBUDCE4.1 vector expressing 5-LO activating protein (FLAP) were utilized (25,26) to screen compounds for 5-LO inhibition. .

For cell stimulation of 5-LO products, transfected HEK293 cells were collected following trypsinization, washed and the cell pellet was resuspended in Hank's balanced salt solution (HBSS) (Lonza, Walkerville, MD) containing 1.6 mM $CaCl_2$ at a concentration of 5 x 10^5 cells mL^{-1}. Cells were pre-incubated with each test compound at 1 µM for 5 min at 37 ^oC. Cells were then stimulated for 15 minutes at 37 ^oC with the addition of 10 µM calcium ionophore A23187 (Sigma–Aldrich, Oakville, ON, Canada) and 10 µM arachidonic acid (Cayman Chemical, Ann Arbor, MI). Stimulations were stopped by the addition of 0.5 volume of cold MeOH:CH_3CN (1:1) containing 50 ng of PGB_2 as internal standard and samples were stored at -20^oC until processing on octadecyl (C18) columns and analysis by RP-HPLC as described previously (25,26). Data are expressed as means ± SEM of 3 independent experiments, each performed in duplicate (fig. 3).

Stimulation of human polymorphonuclear leukocytes (PMNL) for 5-LO products

Human PMNL prepared from peripheral blood as described (23) were suspended in HBSS containing 1.6 mM $CaCl_2$ (10^7 cells/ml) and pre-incubated with test compounds for 5 min at 37 ^oC in the presence of 1 U/ml of adenosine deaminase (Sigma-Aldrich, Oakville, On, Canada). Cells were then stimulated for 15 min at 37^oC with 1 µM thapsigargin (Sigma-Aldrich) and 10 µM AA (Cayman Chemical, Ann Arbour, MI) as previously described (23). Reactions were stopped, processed and analysed by RP-HPLC as indicated above. Data are expressed as means ± SEM of 3 independent experiments, each performed in duplicate (fig. 4). Initial screening of compounds were performed in the presence of 1 µM of test compounds followed by concentration-response assays of selected compounds at 0.1, 0.3, 1.0 and 3.0 µM.

Molecular docking

Molecular docking was done with AutoDock Vina 1.1.2 on 5-LO (PDB code: 3O8Y) following a recently described procedure (27) outlined in "Design, synthesis and evaluation of semi-synthetic triazole-containing caffeic acid analogues as 5-lipoxygenase inhibitors." by Daniela De Lucia et al. For optimization, the MMFF94 force field was used. Results were analyzed with Maestro 10.4 (27)

Results and discussion

Chemistry

Caffeic propargyl esters (**5a-h**) (scheme 1) were synthesized starting from caffeic acid **2**, which was acetylated (26) to yield diacetylcaffeic acid (**9**). Acetylated propargyl caffeate (**10**) was obtained by condensing diacetylcaffeoyl chloride, obtained by refluxing (**9**) in excess thionyl chloride and catalytic DMF, with propargyl alcohol in pyridine/CH_2Cl_2. Compounds (**11b-h)**, acetylated precursors of the final esters, were obtained in moderate to good yields through optimized Sonogashira cross coupling conditions with propargyl ester **10** and the appropriate aryl iodide in the presence of catalytic $PdCl_2(PPh_3)_2$ and copper iodide in dry and degassed DMF/NEt_3. Dimer (**11i)** was obtained in similar conditions with 1,4-diiodobenzene as the aryl iodide and a slight excess of compound (**10)**. Deacetylation of compounds (**10**) and **(11b-i**) to afford caffeic esters (**4a-i**) was performed with guanidinium hydrochloride and triethylamine in MeOH/CH_2Cl_2, thus generating the expected phenolic compounds (28).

Scheme 1. Synthetic route to propargyl esters (**4a-i)**. Reagents: i) AcOAc, DMAP, pyridine, 0 ^oC to rt overnight; ii) $SOCl_2$, DMF_{cat}, reflux 3h then $HCCCH_2OH$, pyridine/CH_2Cl_2, rt overnight ; iii) aryl iodide, $PdCl_2(PPh_3)_2$, CuI, DMF/NEt_3, rt overnight; iv) guanidinium HCl, NEt_3, MeOH/CH_2Cl_2, rt 4h.

Caffeic allyl esters **5a-i** (scheme 2) were synthesized in a similar fashion to compounds (**4a-i**). Acetylated esters (**13b-h**) were obtained in moderate to good yields through optimized Heck cross coupling conditions by reacting allyl ester (**12**) with the required aryl iodides in the presence of catalytic $Pd(OAc)_2$ and Ag_2CO_3 in refluxing benzene. Dimer (**13i**) was obtained in similar conditions with 1,4-diiodobenzene as the aryl iodide and a slight excess of compound (**12**). Deacetylation of compounds (**12**) and **(13b-i**) to afford caffeic esters (**5a-h**) was performed with guanidinium hydrochloride and triethylamine in MeOH/CH_2Cl_2, identical to the propargyl derivatives (28).

Scheme 2. Synthetic route to allyl esters (**5a-i)**. Reagents: i) $SOCl_2$, DMF_{cat}, reflux 3h then CH_2=$CHCH_2OH$, pyridine/CH_2Cl_2, rt overnight; ii) aryl iodide, $Pd(OAc)_2$, $AgCO_3$, PhH, reflux overnight; iii) guanidinium HCl, NEt_3, MeOH/CH_2Cl_2, rt 4h.

Caffeoyl alcohol (**6**) and allyl ethers (**7a**) and (**7b**) (scheme 3) were synthesized starting from caffeic acid **2**, which was esterified in MeOH with concentrated sulphuric acid, yielding ester (**14**). Methyl caffeate (**14**) was then protected as the tetrahydropyranyl ester (**15**) through PPTS catalyzed acetal formation in DHP. The resulting mixture of diastereoisomers was quantitatively reduced to the tetrahydropyranylated allylic alcohol (**16**) by DIBAL-H in dry toluene at -78.

Allyl ethers (**17a-b**) were obtained through Williamson conditions with (**16**) and NaH in DMF to generate the alkoxide, which was subsequently reacted with either benzyl bromide or (3-bromopropyl)benzene to produce the expected ethers in good yields. Deprotection of tetrahydropyranylated compounds (**16**) and (**17a-b**) by methanolysis in the presence of PPTS yielded the caffeoyl alcohol (**6**) as well as allyl ethers (**7a**) and (**7b**) in good yields.

Scheme 3. Synthetic route to caffeoyl alcohol **6** and allylic ethers (**7a-b**). Reagents: i) MeOH, H_2SO_4, reflux 4h; ii) DHP, PPTS, reflux 1.5h; iii) DIBAL-H, $PhCH_3$, -78 ^oC 2h; iv) NaH, $PhCH_2Br$ or $PhCH_2CH_2CH_2Br$, DMF, 0 ^oC 2h; v) (**6**) from (**16**), (**7a-b**) from (**17a-b**); ppts, $MeOH/CH_2Cl_2$, rt 4h.

Tetrahydropyranylated caffealdehyde (**19a**) (scheme 4) was conveniently synthesized by oxidation of allyl alcohol (**16**) with excess MnO_2 in CH_2Cl_2 (29). Acetylated caffeoyl ketones (**19b-e**) were obtained in two steps from the previously synthesized tetrahydropyranylated methyl ester (**15**). Initial conversion of (**15**) to the Weinreb amide (**18**) with *N,O*-dimethylhydroxylamine hydrochloride and isopropylmagnesium chloride (30) was followed by Grignard reaction with the required organomagnesium reagents to yield the desired ketones in moderate yields. Deprotection of tetrahydropyranylated compounds (**19a-e**) by methanolysis in the presence of PPTS yielded the caffealdehyde (**8a**) as well as caffeoyl ketones (**8b-e**) in excellent yields.

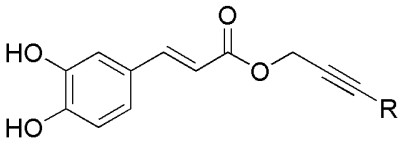
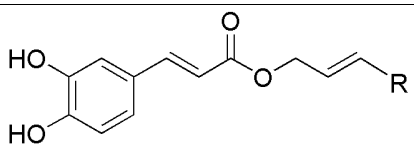
Scheme 4. Synthetic route to caffeoyl aldehyde **8a** and ketones (**8b-e**). Reagents: i) DIBAL-H, $PhCH_3$, -78 ^oC 2h; ii) CH_3NHOCH_3 - HCl, i-prMgCl, THF, -20 ^oC 35 mins; iii) (**19a**) from (**16**), MnO_2, CH_2Cl_2, rt overnight; iv) (**19b-e**) from (**18**), R_1MgBr, THF, O ^oC 1h; v) ppts, $MeOH/CH_2Cl_2$, rt 4h.

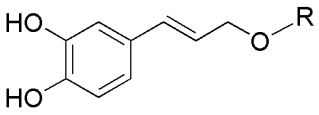
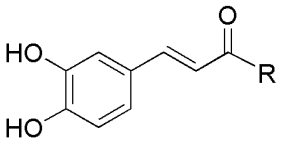
Radical scavenging activity

Radical scavenging activities of synthesized caffeoyl propargyl esters (**4a-i**), allyl esters (**5a-i**), alcohol/ethers (**6**, **7a** and **7b**) and aldehyde/ketones (**8a-e**) were assayed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a stable radical (20) and are expressed as IC_{50} concentrations in table 1. As was expected of catechol-containing molecules, all compounds possessed considerable radical scavenging ability with most having IC_{50} values in the range of 10 – 15 µM, similar to radical scavenging activities of caffeic acid (**2**, 15.3 µM) and CAPE (**3**, 11.9 µM). Little variation in radical scavenging activity of monovalent esters (**4a-h** and **5a-h**) was found, both between related propargyl or allyl esters as well as between analogous esters of both series. This finding underlines the fact that radical scavenging activity stems from the catechol moiety of caffeoyl derivatives and is relatively insensitive to modification that do not directly alter this group (31-33). Logically, the radical scavenging activities of caffeoyl ester dimers (**4i** and **5i**), containing two catechol moieties and thus four phenolic hydroxyl groups, is roughly twice as high as that of monovalent caffeoyl esters (**4a-h**) and (**5a-h**).

Caffeoyl alcohol (**6**) and ethers (**7a-b)** again had IC_{50} values in the 10-15 μM range, similar to their related ester analogs. These findings were surprising as the conjugated electron withdrawing ester groups of compounds (**4a-h**) and (**5a-h**) have long range influences on the catechol rings electron density, as is made evident in ^{1}HNMR analysis of compounds. For example, the phenolic hydrogens of caffeoyl ester (**4a**) have significantly higher chemical shifts than those of allyl alcohol (**6**) (9.40 ppm and 8.90 ppm in DMSO-d_6, respectively). Similarly, all (**3**) aromatic catechol hydrogens of ester (**4a**) are significantly downshifted when compared to those of allyl alcohol (**6**), suggesting reduced electron density due to the α,β-unsaturated ester. Nonetheless, no drastic change is observed in radical scavenging activity, though the lipophilic benzyl and phenpropyl allyl ethers are somewhat more potent radical scavengers than the more hydrophilic allyl alcohol. Caffeoyl aldehyde (**8a**), caffeoyl phenyl ketone (**8b**) and caffeoyl benzyl ketone (**8c**) showed similar radical scavenging capacities with IC_{50} of about 10 μM. However, elongation of the linker separating the carbonyl from the terminal aryl group, as in compound (**8d**: IC_{50} = 19.3 μM) and (**8e**: IC_{50} = 28.2 μM), resulted in a steady decrease in potency.

Table 1. Radical scavenging activity of synthesized caffeoyl derivatives. Data are expressed as means ± SD of 3 independent experiments, each performed in triplicate.

Compound		R	Radical scavenging IC_{50} (μM) [SD]
4a		H	11.1 ± 1.0
4b		Ph	12.7 ± 0.2
4c		4-CH_3-Ph	11.5 ± 0.9
4d		4-CH_3O-Ph	13.7 ± 0.4
4e		4-NO_2-Ph	10.6 ± 0.2
4f		4-F-Ph	10.7 ± 1.9
4g		1-naphtyl	13.7 ± 0.8
4h		4-Ph-Ph	15.0 ± 2.2
4i		4-(C≡CCH_2OCaffeoyl)-Ph	5.6 ± 0.33
5a		H	12.3 ± 0.2
5b		Ph	12.4 ± 0.1
5c		4-CH_3-Ph	13.1 ± 0.3
5d		4-CH_3O-Ph	11.6 ± 0.1

5e		4-NO_2-Ph	12.3 ± 0.6
5f		4-F-Ph	12.31 ± 0.04
5g		1-naphtyl	13.0 ± 0.6
5h		4-Ph-Ph	12.03 ± 0.01
5i		4-(CH=$CHCH_2$OCaffeoyl)-Ph	6.1 ± 0.2
6		H	15.20 ± 0.01
7a		CH_2Ph	12.0 ± 0.14
7b		$CH_2CH_2CH_2$Ph	11.3 ± 0.3
8a		H	10.7 ± 0.2
8b		Ph	8.97 ± 1.2
8c		CH_2Ph	11.6 ± 2.1
8d		CH_2CH_2Ph	19.3 ± 2.8
8e		$CH_2CH_2CH_2$Ph	28.2 ± 2.6
2*	Caffeic acid	OH	15.3
3*	CAPE	OCH_2CH_2Ph	11.9

*Leblanc et al., 2012

Inhibition of 5-LO product synthesis in whole HEK293 cells.

Inhibition of leukotriene biosynthesis was assayed first in intact HEK293 cells that are stably transfected with 5-LO and FLAP, thereby possessing all of the necessary cellular machinery for LTs biosynthesis. This model serves as a highly reproducible model of 5-LO product biosynthesis in which compounds can be preliminarily screened for anti-LTs activity before moving on to more complex systems (25). All compounds were assayed at 1 µM, including known inhibitors CAPE (**3**) and Zileuton (**1**) whose inhibitory potencies were used as reference points (figure 3). As can be seen in the upper left of figure 3, among the propagyl caffeoyl esters of caffeic acid (**4a-i**), only compound (**4i**), a dimer type compound bearing two caffeoyl residues, out performed known 5-LO inhibitor CAPE (**3**) with roughly 80% inhibition of LTs biosynthesis in the HEK293 cell model. One could hypothesize that since compound (**4i**) is the most potent radical scavenger amongst this series (Table 1), a trend may exist between radical scavenging activity and LTs biosynthesis inhibition. However, all other inhibitors in this series (**4a-h**) only possess one caffeoyl residue and have fairly similar radical scavenging activities between them, though they possess markedly different anti-LTs activity, a finding which is supported by past studies (23, 34).

Figure 3. Inhibition of 5-LO products synthesis by synthetic CAPE analogs (1 μM) in whole HEK293 cells.

For example, CAPE (**3**) and compounds (**4e**) and (**4f**) have near identical IC_{50} values in the radical scavenging assays (table 1), however CAPE (**3**) and compound (**4e**) show much better 5-LO inhibition than compound (**4f**). This discrepancy between radical scavenging capacity and anti-LTs activity is echoed in the anti-LTs activity of related caffeoyl esters (**5a-e**), alcohol/ethers (**6, 7a-b**) and aldehyde/ketones (**8a-e**). Notably, ketones (**8b-e**), which have progressively lower radical scavenging activity as the alkyl bridge linking their terminal phenyl group is extended, retain near identical anti-LTs activity with CAPE (**3**) in this assay.

Interestingly, some analogous caffeoyl esters have markedly different 5-LO inhibition activity. Propargyl esters (**4b**: R = Ph) and (**4f**: R = 4-F-Ph), for example, possess much lower anti-LTs activity than their related allyl esters (**5b**) and (**5f**). These compounds differ only by the presence of a *trans* alkene vs linear alkyne link between the caffeoyl ester and terminal aromatic group, highlighting the importance of geometry in these compounds for inhibition.

Caffeoyl ethers (**7a-b**), a class of caffeic acid derived 5-LO inhibitors which have not previously been tested for anti-LTs activity, outperformed caffeoyl alcohol (**6**), CAPE (**3**) and Zileuton (**1**) in HEK293 cell based assays. That lipophilic ethers (**7a-b**) were better inhibitors than the more polar alcohol (**6**) may highlight the importance of polarity in inhibitory activity, as it has previously been found that caffeic acid itself is a very poor inhibitor of 5-LO in cell based assays (23). As mentioned above, caffeoyl ketones (**7b-e**) were found to be roughly equipotent with CAPE for 5-LO inhibition in HEK293 cells.

Compounds that approached or surpassed the anti-LTs activity of CAPE (**3**) were selected for further screening in human PMNL.

Inhibition of 5-LO product synthesis in thapsigargin-stimulated human PMNL.

To further probe inhibitory activity of lead compounds selected from preliminary HEK293 screenings (fig. 3), 5-LO inhibition assays were undertaken in stimulated human PMNL in the presence of 1 μM of inhibitors. 5-LO is highly expressed in PMNL and these cells are important physiological producers of LTB_4 (23, 35).

As can be seen in figure 4, propargyl esters (**4c**) and (**4d-e**), which possessed similar potency in HEK293 cells, differ significantly in PMNL based assays. 4-Me-phenyl substituted (**4c)** is significantly less active than (**4d-e**) (4-MeO-Phenyl and 4-NO_2-Phenyl, respectively), though these compound differ in structure only in the presence of the 4-substituent of their terminal phenyl group. These subtle structural changes may influence availability or cell permeation of inhibitors in the more complex PMNL versus HEK293 cells. Impressively, dimer ester (**4i**) induced almost complete inhibition of LTs biosynthesis at 1 μM, widely surpassing the activity of known inhibitor CAPE (**3**).

As can be seen in figure 4, inhibition results for allyl esters (**5b-f, 5i**) were similar their propargyl counterparts. Specifically, compounds (**5c**) and (**5d-e**), which differ from each other in the same way as esters (**4c**) and (**4d-e**) (R = 4-Me-Phenyl vs 4-MeO-Phenyl and 4-NO_2-Phenyl, respectively), show a nearly identical inhibition pattern to their propargyl counterparts, (**5d-e**) outperforming (**5c**). Again, dimer compound (**5i**) was the best inhibitor in this class, surpassing CAPE (**3**), though was not as potent as its propargyl analog (**4i**).

Impressively, novel caffeoyl ethers (**7a-b**) produced near total inhibition of LTs biosynthesis in PMNL based assays, suggesting these lipophilic ethers may be a privileged structure for 5-LO inhibition in a physiological setting.

Ketones (**8b-e**) became progressively more potent as the alkyl chain linking the caffeoyl ketone moiety to the terminal phenyl group became longer (with 0, 1, 2 or 3 methylene units respectively separating carbonyl and phenyl groups).

Figure 4. Inhibition of 5-LO products synthesis by selected synthetic CAPE analogs (1 μM) in thapsigargin stimulated human PMNL.

It is interesting to note that the inverse pattern is apparent in radical scavenging activity, with compound (**8b**) being most potent with diminishing activity up to compound (**8e**).

In order to better analyse the anti-LTs activity of these compounds, selected inhibitors with LTs biosynthesis inhibition of at least 50% at 1 μM in PMNL (all compounds from figure 4 except **4c, 5c, 5f, 6, 8b** and **8c**) were screened further through concentration-response studies in stimulated PMNL (table 2).

Selected compounds were assayed between 0.1 – 3 μM in order to obtain dose response curves, from which were calculated IC_{50} values. All compounds inhibited 5-LO in a concentration-dependent manner and showed promising activities, with IC_{50} values ranging from 0.18-1.03 μM.

Table 2. IC_{50} values of selected inhibitors.

Compound	Human PMNL (μM) [SEM]	Compound	Human PMNL (μM) [SEM]
Zileuton (**1**)	3.0 [0.2]	**5e**	0.88 [0.05]
CAPE (**3**)	0.99 [0.08]	**5i**	0.61 [0.04]
4d	0.83 [0.07]	**7a**	0.43 [0.04]
4e	0.68 [0.04]	**7b**	0.18 [0.01]
4i	0.36 [0.03]	**8d**	0.84 [0.05]
5b	1.03 [0.02]	**8e**	0.64 [0.05]
5d	0.73 [0.04]		

Most compounds outperformed CAPE **3** and all possessed IC_{50} values significantly lower than the clinical 5-LO inhibitor Zileuton **1** in PMNL, with propargyl ester dimer **4i**, allyl dimer **5i** and caffeoyl ether **7a-b** being roughly 8-, 5-, 7- and 16-fold more potent than Zileuton in this model.

Molecular Docking

Molecular docking was done on a modified 5-LO (PDB code: 3O8Y) protein with various ligands. Zileuton was docked as reference. Its binding energy was determined with AutoDock Vina of -6.6 kcal/mol and forms two hydrogen bonds with Leu420 (OH···O, d = 2.48 Å: NH···O, d = 1.64 Å). For molecule (**7b**), the affinity energy was -8.2 kcal/mol with one hydrogen bond (OH···O, d = 2.24 Å) with Leu420. Both molecules take a similar position as can be seen in figure 5. Ligand (**7b**) positions itself over most of the length of a docked arachidonic acid molecule.

Figure 5. Results of AutoDock Vina docking displayed in Maestro of Zileuton (left) and **7b** (right)

Comparing the best inhibitor, **7b**, with CAPE (**3**) and less flexible analogues (**4b** and **5b**) shows that the flexibility of the ester part is essential 5-LO inhibition. Even these analogues have similar affinity as **7b**, they were clearly less active (table 2 and 3).

Table 3. Molecular docking data of selected inhibitors.

Compound	Affinity (kcal/mol)	Hydrogen bonding	π-π Interaction
(S)-Zileuton*	- 6.5	-	-
(R)-Zileuton*	- 6.6	Leu420 x 2	Phe421 x2
CAPE (**3**)	- 8.5	-	His367
4b	- 8.2	-	Phe177, His367
5b	- 8.2	Asn407	-
7b	- 8.2	Leu420	His372
8e	- 8.7	-	Phe177, His367

***** Zileuton is formulated as a racemic mixture.

Hydrogen bond with Leu420 seems to be necessary for 5-LO inhibition, **7b** which formed this bond with this specific amino acid was our best 5-LO inhibitor (IC_{50}: 0.18 µM). The same bond was also present when Zileuton was modeled with 5-LO (table 3).

π-π Interaction with His372 was also identified as crucial for a good inhibition of 5-LO. This interaction was only found with **7b** and not with any other molecules (table 3). Hydrogen bond with Asn407 and π-π interactions with Phe421, Phe 177, and His367 do not seem to be crucial for a strong 5-LO inhibition. The absence of the hydrogen bond with Leu420 as well as π-π interaction with Hist372 may explain why the ketone **8e** (IC_{50}: 0.64 µM) was slightly less active than the ether **7b** (IC_{50}: 0.18 µM) (table 3).

Further molecular modeling studies should confirm these hypotheses to better understand the mode of action of such inhibitors and even for further structural refinement of new 5-LO inhibitors.

Conclusion

In summary, 26 caffeic acid phenethyl ester analogs, including propargyl (**4a-i**) and allyl (**5a-i)** esters of caffeic acid as well as caffeoyl alcohol (**6**), ethers (**7a-b**), aldehyde (**8a**) and ketones (**8b-e**) were designed, synthesized and tested for their radical scavenging and anti-LTs activity. All compounds were

found to possess good radical scavenging activity, with IC_{50} values proving relatively insensitive to modifications not affecting the catechol ring of inhibitors. Compounds were submitted to an initial screening for 5-LO inhibition in 5-LO/FLAP-transfected HEK293 cells before being assayed in human polymorphonuclear leukocytes (PMNL). Interestingly, no correlation between 5-LO inhibition and radical scavenging activity were evident in these assays. Though aromatic substitution patterns in assayed allyl and propargyl esters influenced inhibitory activity, many proved no better than CAPE (**3**) in potency, though most surpassed Zileuton (**1**) in PMNL. Dimer caffeoyl esters (**4i**) and (**5i**) however proved to be excellent inhibitors, along with caffeoyl ethers (**7a-b**) which showed near complete inhibition of LTs biosynthesis at assayed concentrations. Further structural investigation of these scaffolds, particularly the caffeoyl ethers which are a novel class of 5-LO inhibitors, are currently underway along with pharmacodynamics studies in more complex biological models.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Supplementary material

Supporting information including synthetic details and spectroscopic analysis of compounds **4b-i**, **5a-i**, **7a-b**, **8a-e, 11c-h** and **13c-h** is available online.

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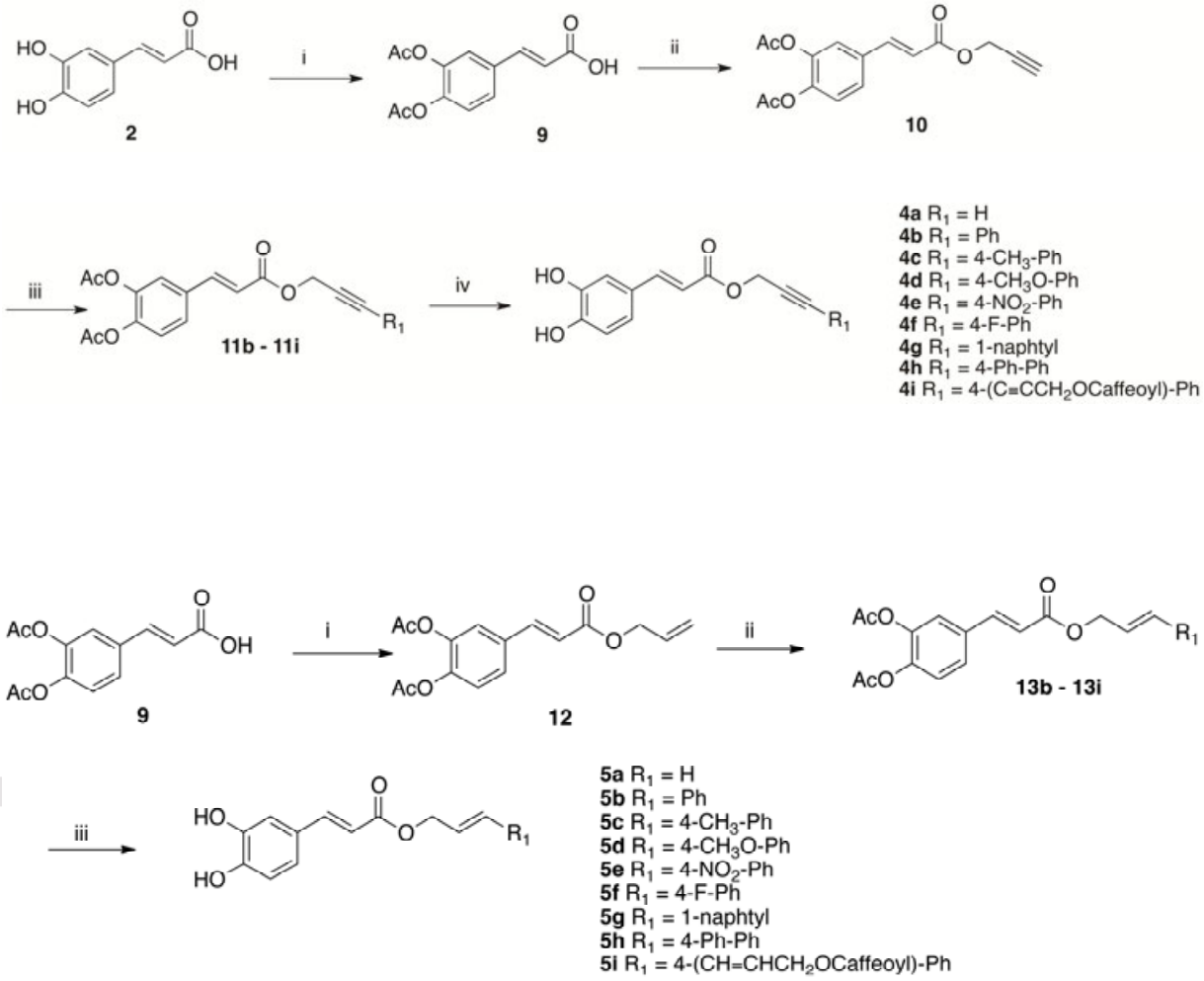
References

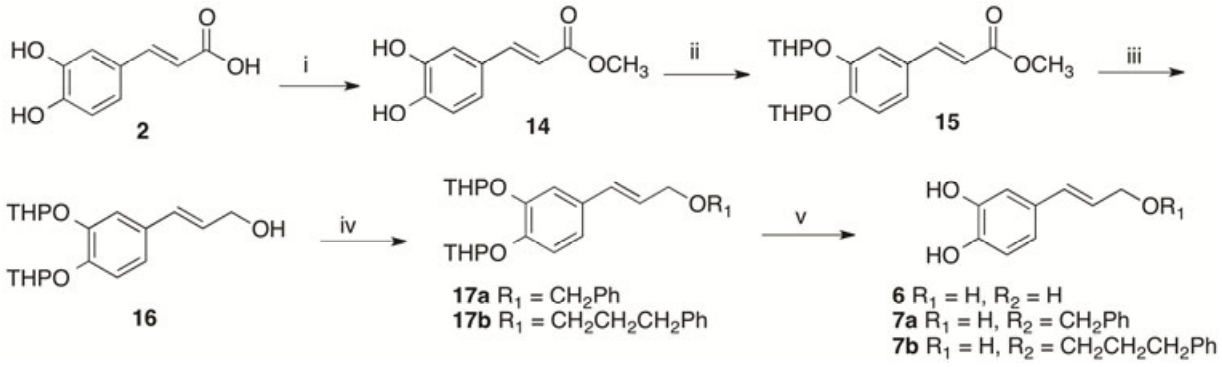
1. Peters-Golden, M., & Henderson, W. R. Jr. (2007). Leukotrienes. New England Journal of Medecine, 357(18), 1841-1854.
2. Chen, X., Li, N., Wang, Su, W. N., Hong, J., Jiao, X., Krasna, M. J., Beer, D. G., & Yang C. S. (2003). Leukotriene A4 hydrolase in rat and human esophageal adenocarcinomas and inhibitory effects of bestatin. The Journal of the National Cancer Institute, 95(14), 1053-1061.
3. Ghosh, J., & Myers, C. E (1998). Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. Proceedings of the National Academy of Sciences of the

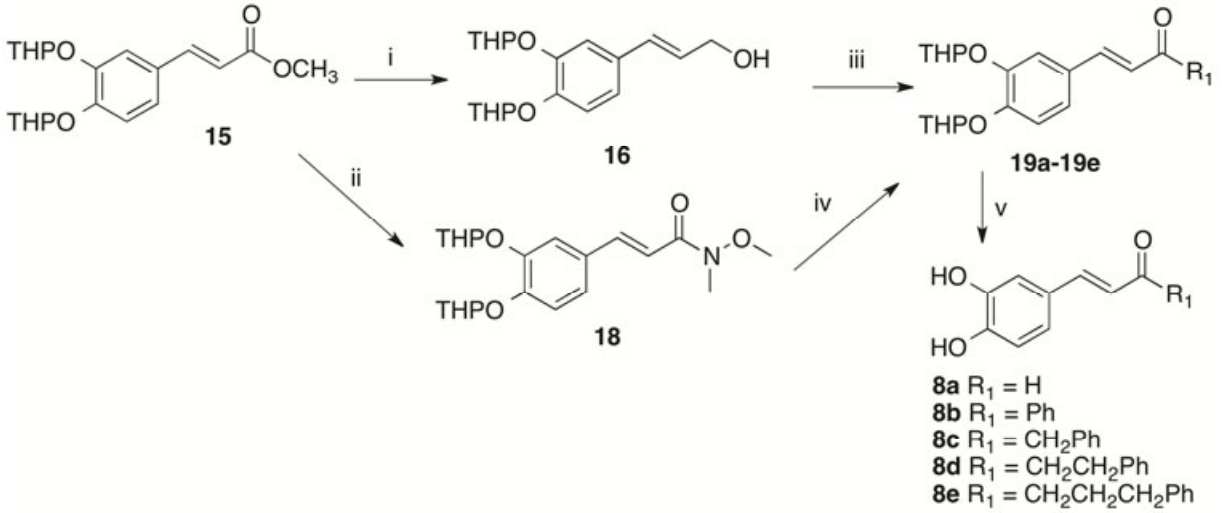
United States of America, 95(22), 13182–13187.
4. Rioux, N., & Castonguay, A. (1998). Inhibitors of lipoxygenase: a new class of cancer chemopreventive. Carcinogenesis, 19(8), 1393–1400.
5. Sun, Z., Sood, S., Li, N., Ramji, D., Yang, P., Newman, R. A., Yang, C. S., & Chen, X. (2006). Involvement of the 5-lipoxygenase/leukotriene A4 hydrolase pathway in 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamster cheek pouch, and inhibition of carcinogenesis by its inhibitors. Carcinogenesis, 27(9), 1902-1908.
6. Pergola, C., & Werz, O. (2010). 5-Lipoxygenase inhibitors: a review of recent developments and patents. Expert Opinion on Therapeutic Patents, 20(3), 355-375.
7. Werz, O., & Steinhilber, D. (2005). Pharmacological intervention with 5-lipoxygenase: new insights and novel compounds. Expert Opinion on Therapeutic Patents, 15(5), 505-519.
8. Berger, W., De Chandt, M. T., & Cairns, C. B. (2007). Zileuton: clinical implications of 5-Lipoxygenase inhibition in severe airway disease. International Journal of Clinical Practice, 61(4), 663–676.
9. Garcia-Marcos, L., Schuster, A., & Perez-Yarza, E. G. (2003). Benefit-risk assessment of antileukotrienes in the management of asthma. Drug Safety, 26(7), 483–518.
10. Liu, M. C., Dube, L. M., & Lancaster, J. (1996). Acute and chronic effects of a 5-lipoxygenase inhibitor in asthma: a 6-month randomized multicenter trial. Zileuton Study Group. Journal of Allergy and Clinical Immunology, 98(5 Pt 1), 859-871.
11. Bankova, V., Dyulgerov, Al., Popov, S., & Marekov, N. (1987). A GC/MS study of the propolis phenolic constituents. Zeitschrift fuer Naturforschung, C: Journal of Biosciences, 42(1-2), 147-151.
12. Sawicka, D., Car, H., Borawska, M. H., & Niklinski, J. (2012). The anticancer activity of propolis. Folia Histochemica et Cytobiologica, 50(1), 25–37.
13. Watanabe, M. A. E., Amarante, M. K., Conti, B. J., & Sforcin, J. M. (2011). Cytotoxic constituents of propolis inducing anticancer effects: a review. Journal of pharmacy and pharmacology, 63(11), 1378–1386.
14. Akyol, S., Ozturk, G., Ginis, Z.; Armutcu, F.; Yigitoglu, M. R., & Akyol, O. (2013). In Vivo and In Vitro Antıneoplastic Actions of Caffeic Acid Phenethyl Ester (CAPE): Therapeutic Perspectives. Nutrition and Cancer, 65(4), 515–526.
15. Grunberger, D., Banerjee, R., Eisinger, K., Oltz, E. M., Efros, L., Caldwell, M., Estevez, V., & Nakanishi, K. (1988). Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Experientia, 44(3), 230–232.
16. Lin, H.-P., Lin, C.-Y., Huo, C., Su, L.-C., & Chuu, C.-P. (2012). Anticancer Effect of Caffeic Acid Phenethyl Ester. Pharmacologia, 3(1), 26-30.
17. Najafi, M. F., Vahedy, F., Seyyedin, M., Jomehzadeh, H. R., & Bozary, K. (2007). Effect of the water extracts of propolis on stimulation and inhibition of different cells. Cytotechnology, 54(1), 49–56.
18. Koshihara, Y., Neichi, T., Murota, S.-I., Lao, A.-N., Fujimoto, Y., & Tatsuno T. (1983). Selective inhibition of 5-lipoxygenase by natural compounds isolated from Chinese plants, Artemisia rubripes Nakai. FEBS letters, 158(1), 41–44.
19. Koshihara, Y., Neichi, T., Murota, S.-I., Lao, A.-N., Fujimoto, Y., & Tatsuno, T. (1984). Caffeic acid is a selective inhibitor for leukotriene biosynthesis. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 792(1), 92–97.

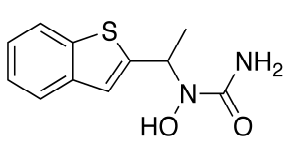
20. Lin, C. F., Chang, T. C., Chiang, C. C., Tsai, H. J., & Hsu, L. Y. (2005). Synthesis of Selenium-Containing Polyphenolic Acid Esters and Evaluation of Their Effects on Antioxidation and 5-Lipoxygenase Inhibition. Chemical & Pharmaceutical Bulletin, 53(11), 1402-1407.
21. Mirzoeva, O. K., & Calder, P. C. (1996). The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostaglandins, Leukotrienes and Essential Fatty Acids, 55(6), 441-449.
22. Sud'ina, G. F., Mirzoeva, O. K., Pushkareva, M. A., Korshunova, G. A., Sumbatyan, N. V., & Varfolomeev, S. D. (1993). Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Letters, 329(1-2), 21-24.
23. Boudreau, L. H., Maillet, J., Leblanc, L. M., Jean-François, J., Touaibia, M., Flamand, N., & Surette, M. E. (2012). Caffeic Acid Phenethyl Ester and Its Amide Analogue Are Potent Inhibitors of Leukotriene Biosynthesis in Human Polymorphonuclear Leukocytes. PLoS ONE, 7(2), e31833.
24. Pontiki, E., & Hadjipavlou-Litina, D. (2008). Lipoxygenase inhibitors: a comparative QSAR study review and evaluation of new QSARs. Medical Research Reviews, 28(1), 39–117.
25. Boudreau, L. H., Picot, N., Doiron, J., Villebonnet, B., Surette, M. E., Robichaud, G. A., & Touaibia, M (2009). Caffeoyl and cinnamoyl clusters with anti-inflammatory and anti-cancer effects. Synthesis and structure-activity relationship. New Journal of Chemistry, 33(9), 1932-1940.
26. Doiron, J., Boudreau, L. H., Picot, N., Villebonet, B., Surette, M. E., & Touaibia, M. (2009). Synthesis and 5-Lipoxygenase Inhibitory Activity of New Cinnamoyl and Caffeoyl Clusters. Bioorganic and Medicinal Chemistry Letters, 19(4), 1118-1121.
27. De Lucia, D., Lucio, O. M., Musio, B., Bender, A., Listing, M., Dennhardt, S., Koeberle, A., Garscha, U., Rizzo, R., Manfredini, S., Werz, O., Ley, S. V. (2015). Design, synthesis and evaluation of semi-synthetic triazole-containing caffeic acid analogues as 5-lipoxygenase inhibitors. European Journal of Medicinal Chemistry, 101(28), 573-583. Schrödinger Release 2015-4: Maestro, version 10.4, Schrödinger, LLC, New York, NY, 2015.
28. Kunesch, N., Meit, C., & Poisson, J. (1987). Utilisation de la guanidine comme agent désacétylant sélectif: une method de désacétylation instantanée applicable aux sucres. Tetrahedron Letters, 28(31), 3569-3572.
29. Chen, F., Parvathi, K., Blount, J. W., Dixon, R. A. (2001). Chemical syntheses of caffeoyl and 5-OH coniferyl aldehydes and alcohols and determination of lignin O-methyltransferase activities in dicot and monocot species. Phytochemistry, 58(7), 1035–1042.
30. Williams, J. M., Jobson, R. B., Yasuda, N., Marchesini, G, Dolling, U.-H., & Grabowski, E. J. J. (1995). A new general method for reparation of N-methoxy-N-methylamides. Application in direct conversion of an ester to a ketone. Tetrahedron Letters, 36(31), 5461-5464.
31. Chen, J. H., & Ho, C.-H. (1997). Antioxidant Activities of Caffeic Acid and Its Related Hydroxycinnamic Acid Compounds. The Journal of Agricultural and Food Chemistry, 45(7), 2374–2378.
32. Torres de Pinedo, A., Penalver, P., Morales, J. C. (2007). Synthesis and evaluation of new phenolic-based antioxidants: Structure–activity relationship. Food Chemistry, 103(1), 55-61.
33. LeBlanc, L. M., Paré, A. F., Jean-François, J., Hébert, M. J. G., Surette, M. E., & Touaibia, M. (2012). Synthesis and Antiradical/Antioxidant Activities of Caffeic Acid Phenethyl Ester and Its Related Propionic, Acetic, and Benzoic Acid Analogues. Molecules, 17(12), 14637-14650.

34. Doiron, J. A., Métayer, B., Richard, R. R.; Desjardins, D.; Boudreau, L.H., Lesveque, N. A., Jean-François, J., Poirier, S. J., Surette, M. E., Touaibia, M. (2014). Clicked cinnamic/caffeic esters and amides as radical scavengers and 5-lipoxygenase inhibitors. International journal of medicinal chemistry, Vol. 2014, Article ID 931756, 12 pages.
35. Walsh C, E., Waite, B. M., Thomas, M. J., DeChatelet, L. R. (1981). Release and metabolism of arachidonic acid in human neutrophils. Journal of Biological Chemistry, 256(14), 7228–7234.

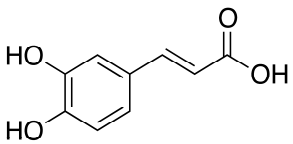




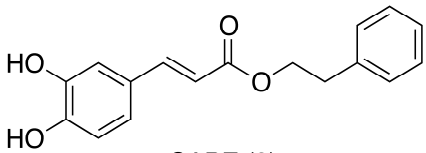




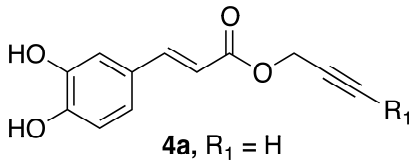
Zileuton (**1**)



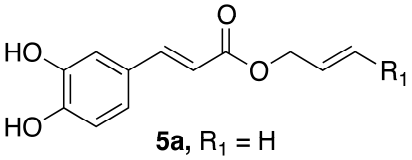
Caffeic acid (**2**)



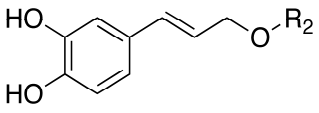
CAPE (**3**)



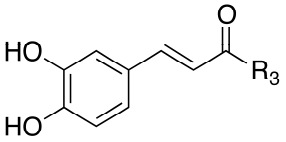
4a, R_1 = H
4b-i, R_1 = Ar



5a, R_1 = H
5b-i, R_1 = Ar



6, R_2 = H
7a-b, R_2 = CH_2Ph, $CH_2CH_2CH_2Ph$)



8a, R_3 = H
8b-e, R_3 = Ph, CH_2Ph, $(CH_2)_2Ph$, $(CH_2)_3Ph$